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My dear Lederberg,

Thank you very much for your letter of August 30. I apologize for not having answered it earlier but I just came back from Holland a few days ago.

With regard to Lettré's experiments with ascites cells,distilled water and mitochondria,I should like to start with the following,at first sight somewhat incoherent facts which we have found on different occasions:

1).A dose of 100 Ehrlich ascites tumor cells leads to ascites development in nearly 100 per cent of the mice.

2). When 100 living cells are mixed with 20 million cells that have been irradiated with 5000 r X-rays in vitro (a lethal dose) and injected,no animals develop ascites tumors. This is due to the fact that this tumor has originated in non-inbred mice,and it is possible to build up an effective immunological protection under certain circumstances against the tumor. The 20 million dead cells have an antigenic effect and the host has time to build up its antibody response before the small viable inoculum had a chance to reach a size where regression would no longer be possible.

3).If experiment 2 is modified in such a way that the number of viable cells in the mixture is gradually increased while the number of dead is maintained constant, one arrives first from complete to only partial growth inhibition, then no inhibition,and finally stimulation! Thus,using 2 million living and 20 million dead cells,their growth is considerably stimulated. It would be most probable to assume that under these conditions no effective immunity can develop during the limited period before the 2 million cells reach killing size and the cells are at the same time stimulated in their growth by some substances released by the dead and dying X-rayed cells.

4).The smallest tumor-cell dose which still produces 100 per cent progressive growth with a particular tumor-host combination is dependent to a rather extraordinary degree on

the protein concentration of the medium in which the cells are suspended prior to inoculation. All balanced saline media without proteins are very harmful while the cells can be protected considerably by the addition of gelatin,serum,embryonic extracts,chromatin fractions,mitochondria,etc.etc. The reason for this seems to be the"destructive swelling" of the cells that occurs in media without proteins. This phenomenon has been studied extensively by Shear between 1920 and 1930. Apparently all what matters to prevent or to reverse destructive swelling (which up to a certain point is reversible) is the amount of proteins or other highmolecular elements in the medium.

Coming back to Lettré,one possible interpretation of his experiments would be the following:

After treatment with distilled water,his cell population is a mixture of a small number of living and a great number of dead cells. Upon injection,the effect produced is the same as in experiment 2). and no tumors develop. Adding mitochondria, the number of cells that remain viable increases as some are reversed on their way to destruction by swelling. This results in a shift between the ratio of living to dead cells as under 3). and he will obtain tumors in some animals which is what seems to happen. That mitochondria play no specific role,is indicated by the effect of nuclear fragments and that of mitochondria from non specific sources.

We have repeated one experiment of Lettré's in this connection,namely injecting mitochondria into developed Ehrlich ascites tumors. He claimed a fairly specific mitotic stimulation in the tumor. We have obtained a very small mitotic increase and there was no difference between mitochondria from the tumor itself,from different other tumors,from mouse liver and from rat liver. An enormous mitotic stimulation occurred if the sucrose used for mitochondrial isolation was not washed out carefully, however. From Lettré's papers it is quite impossible to know how many times his mitochondria were washed before injected and whether the considerable stimulation obtained by him could not be due to traces of sucrose in the material injected.

His most recent paper(Zschr.Krebsf.60:80,1954) describes experiments with P_{32} and shows uptake of P_{32} by tumor cells after injection of labeled mitochondria. Obviously, this also falls far from a demonstration of theuptake of mitochondria as structural units,as P_{32} could be released in a number of ways and taken up by the cells.

Summarizingly,I dont think that Lettré has demonstrated with any conclusiveness uptake of mitochondria as structural elements by cells and neither has he shown any specific "resuscitating" action on cells damaged with distilled water.

There is no need to treat any of these comments confidentially. I should not like to give the impression that the above is the correct explanation,but I dont feel that Lettré has proved his point so far.

With regard to """transduction""" of tumor cells,we have followed the problem on somewhat different lines during the last two years. We did not try to induce tumors from normal tissues as I feel that tumor production is a several-step process and the probability to obtain a positive result under controled conditions is meagre. We tried to change established tumors instead. We have attempted to make them more rapidly growing,or genetically less specific,or less differentiated, or growing in ascites by using various extracts from related tumors that have undergone the same changes spontaneously. As possible "transducing agents",we have applied dead cells, homogenates of dead cells,mitochondria,microsomes,nuclei, and pure DNA,and,in the case of ascites transformation, also ascites serum. We have treated the cells both in vivo and in vitro. In vitro we have followed the conditions of bacterial transformation,inhibited DNAse,added serum,made the cells to divide by adding glucose. The result? Absolutely nothing, so far. We shall continue,as negative results do not mean anything. I should like to ask you whether you know of any cases of transduction with organisms other and,particularly, higher than bacteria?

I continue to follow your really ingenious work with the greatest interest. You really produce one great discovery after the other! Hans Ris told me recently that you now can identify pairing bacteria under the microscope. I should be most grateful if you would continue to send me your reprints and also if you would let me know if you have any suggestions or comments with regard to how such possible transduction experiments should be farther conducted.

Very sincerely yours,

George Klein

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